INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

SYNTHESIS, CHARACTERIZATION AND *IN VITRO* ANTIMICROBIAL EVALUATION OF INDOLINE BASED DERIVATIVES

Dhineshkumar Manoharan [1]*, Kannan Kulanthai [1] and Gnanavel Sadhasivam [1], Thirumalairaj Jeyaraman [2]
[1]Department of chemistry, Government College of Engineering, Salem, Tamil Nadu, India
[2]Department of microbiology, Periyar University Salem, Tamil Nadu, India
*Corresponding Author Email: dhineshkavi2020@gmail.com

Article Received on: 30/03/17 Approved for publication: 29/04/17

DOI: 10.7897/2230-8407.080463

ABSTRACT

New series of indoline carbothioamide based derivatives have been synthesized compounds were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR and LC-ESI. All the synthesized compounds were screened for their antibacterial activity against four different strains specifically *Staphylococcus aureus, Bacillus subtilis Escherichia coli and Klebsiella pneumonia* by the disc well diffusion method. While *anti fungal* was determined against two different strains *Candida albicans* and *Candida glabrata* by the agar well diffusion method. In vitro antimicrobial studies revealed that most of the synthesized compounds showed significant activities as compared to standard drugs chloramphenicol and *fluconazole* respectively.

Keywords: indoline, antimicrobial, ^{1}H-NMR

INTRODUCTION

Heterocyclic compounds and their derivatives are found to be associated with various biological activities[1]. Microbial infections are common problem in all over worldwide. At the present days, the occurrence of fungal and bacterial infection has been noticeably increased which leads to serious health hazards[2]. Consequently, so many researchers have focused their probe efforts on finding new, potent, less toxic antibacterial and antifungal drugs. Whereas a variety indoline the potent basic pharmacodynamic nucleus has been reported to possess a wide variety of biological properties such as antimicrobial[3-6], anticancer[7-10], anti-diabetic[11], anti-inflammatory[12, 13], antitubulin[14], antiperoxidative[15], anticoagulant[16], etc.,

In continuation, the indoline nucleus is incorporated in various natural products such as alkaloids[17]. Encouraged by the above observations and considering the interesting pharmacological profile of indoline, we synthesized N-(4-aminophenyl) indoline-1-carbothioamide scaffold based compounds as an antimicrobial agent.

MATERIALS AND METHODS

Chemistry

The synthetic starting materials such as reagents and solvents were used in analytical grade or the highest quality commercially available and were purchased from Sigma-Aldrich Chemical Co., Merck Chemical Co. Melting points were recorded by labtronics digital melting points apparatus. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 solvent on Bruker 300 MHz spectrophotometer using tetramethylsilane as an internal reference. The apparent resonance multiplicity is described as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), td (triplet of doublet), q (quartet) and m (multiplet). Infrared measurements were recorded in the range 400-4000 cm^{-1} by Perkin Elmer. Mass spectra were recorded on a Thermo LCQ Deca XP MAX at 70eV. Thin layer chromatography (TLC) analysis was carried out on 5 x 20 cm plate coated with silica gel GF$_{254}$.

Synthesis *of N-(4-nitrophenyl) indoline-1-carbothioamide* (1)

To a solution of indoline (10 g, 0.0835 mol) in 200 mL of THF, 4-nitrophenyl isothiocyanate (16.53 g, 0.0918 mol) was added at 0 ^{0}C. The resulting reaction mass was allowed to RT and stirred for 4 h. The reaction mass was concentrated under reduced pressure to give the compound **1** as a yellow solid; Yield 80%; m.p 173-174 ^{0}C; IR (KBr) ν_{max} in cm^{-1} : 3434, 3300 (NH stretch), 3074 (CH stretch, aromatic), 2919, 2850 (CH stretch, aliphatic), 1532, 1319, (NO$_2$ stretch), 1477 (CH$_2$ bend), 1270 (C=S), 909 (C-N stretch) ; ^{1}H-NMR (DMSO-d_6) δ ppm: 3.13 (t, 2H, J = 8.1 Hz), 4.32 (t, 2H, J = 8.2 Hz), 7.05 (td, 1H, J = 0.9, 7.9 Hz), 7.18 (td, 1H, J = 1.2, 8.1 Hz), 7.33 (d, 1H, J = 7.9 Hz), 7.71 (d, 2H, J = 9.9 Hz), 7.80 (d, 1H, J = 8.0 Hz), 8.19 (d, 2H, J = 9.9 Hz), 10.39 (s,1H); ^{13}C-NMR (DMSO-d_6) δ ppm: 26.6, 54.2, 116.0, 121.9, 123.9, 124.1, 125.6, 126.8, 134.3, 142.1, 142.2, 146.8, 177.5; LC/ESI calculated for ($C_{15}H_{13}N_3O_2S$) m/z $[M + H]^+$ 299.07, found m/z 300.0665.

Synthesis of N-(4-aminophenyl) indoline-1-carbothioamide (2)

To a solution of compound 1 (10 g, 0.0338 mol) in 100 mL 12N HCl, SnCl$_2$ (63.88 g, 0.338 mol) was added at RT. The resulting reaction mass was stirred for 3 h. The completion of the reaction was monitored by TLC. The reaction mass was diluted with 350 mL of cold water. The resulting reaction mass was basified to pH 8 with 40% NaOH. The aqueous layer was extracted with ethyl acetate (3 x 150 mL) and water (2 x 30 mL), brine (1 x 300 mL) and dried over anhydrous Na$_2$SO$_4$. The organic layer was concentrated under reduced pressure to afford compound **2**. Brown solid; Yield 72% ; mp 209-210 ^{0}C; IR (KBr) ν_{max} in cm^{-1}

: 3398 (NH stretch), 3055 (CH stretch, aromatic), 2969 (CH stretch, aliphatic), 1610 (NH bend), 1447 (CH_2 bend), 1209 (C=S), 932 (C-N stretch) ; ^{1}H-NMR (DMSO-d_6) δ ppm: 3.08 (t, 2H, J = 8.1 Hz), 4.21 (t, 2H, J = 8.2 Hz), 5.05 (s, 2H), 6.52 (d, 2H, J = 8.7 Hz), 6.95 (t, 3H, J = 7.2 Hz), 7.12 (t, 1H, J = 7.8 Hz), 7.24 (d,1H, J = 7.9 Hz), 8.29 (d, 1H, J = 8.1 Hz), 9.41 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 52.8, 113.3, 116.1, 122.5, 124.9, 126.1, 126.8, 128.5, 133.3, 143.4, 146.3, 178.5; LC/ESI calculated for ($C_{15}H_{15}N_3S$) m/z $[M + H]^+$ 269.100, found m/z 270.0979.

Synthesis of 1-(4-fluorophenyl)-3-(4-(indoline-1-carbothioamido)phenyl)thiourea (3a)

To a solution of compound 2 (0.4 g, 0.0015 mol) in 12 mL THF, 4-fluoro phenyl isothiocyanate (0.300 g, 0.0019 mol) was added at 0 ^{0}C. The reaction mixture was allowed to RT and stirred for 2 h. The progress of the reaction was monitored by TLC. The reaction mass was concentrated under reduced pressure and separated between ethyl acetate (1 x 50 mL) and 2N HCl (1 x 25 mL). The resulting ethyl acetate layer was washed with brine (1 x 40 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate layer was concentrated under reduced pressure to afford compound **3a.** Pal yellow solid; Yield 32%; mp 259-261 ^{0}C; IR (KBr) ν_{max} in cm^{-1} : 3424 (NH stretch), 2926 (CH stretch, aromatic), 2581 (CH stretch, aliphatic), 1590 (NH bend), 1449 (CH_2 bend), 1291 (C-N stretch), 1211 (C=S), 761 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.10 (t, 2H, J = 8.3 Hz), 4.26 (t, 2H, J = 8.1 Hz), 6.99 (t, 1H, J = 7.8 Hz), 7.13-7.15 (m, 3H), 7.29 (d, 1H, J = 8.3 Hz), 7.27-7.50 (m, 6H), 8.09 (d, 1H, J = 8.4 Hz), 9.76 (s, 3H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 53.3, 114.8, 115.1, 123.0, 123.6, 125.2, 126.0, 126.4, 133.7, 135.7, 143.0, 157.8, 160.2,178.2, 179.9; LC/ESI calculated for ($C_{22}H_{19}FN_4S_2$) m/z $[M + H]^+$ 422.1, found m/z 423.0681.

Synthesis of 1-(4-(indoline-1-carbothioamido) phenyl)-3-(3-methoxy phenyl)urea (3b)

To a solution of compound 2 (0.5 g, 0.0018 mol) in 15 mL THF, 3-methoxy phenyl isocynate (0.322 g, 0.0024 mol) was added at 0 ^{0}C. The reaction was carried out at RT and stirred for 1 h. The completion of reaction was monitored by TLC. To the resulting residue, diethyl ether was added and the solid was filtered off. The resulting white solid was dried under vacuum to afford compound **3b**. Yield 76% ; mp 246-248 ^{0}C ; IR (KBr) ν_{max} in cm^{-1} : 3287 (NH stretch), 2950 (CH stretch, aromatic), 2838 (CH stretch, aliphatic), 1649 (C=O), 1558 (NH bend), 1491 (CH_2 bend), 1291 (C-N amide), 1230 (C=S), 1160 (C-O), 763 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.10 (t, 2H, J = 8.1 Hz), 3.70 (s, 3H), 4.27 (t, 2H, J = 8.1 Hz), 6.52 (dd, 1H, J = 2.1, 8.1 Hz), 6.92-7.00 (m, 2H), 7.11-7.27 (m, 6H), 7.43 (d, 2H, J = 8.7 Hz), 8.23 (d, 1H, J = 8.2 Hz), 9.41 (d, 2H, J = 9.1 Hz), 9.67 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 53.1, 54.8, 103.5, 106.8, 110.1, 116.1, 117.6, 122.8, 125.0, 125.8, 126.2, 129.4, 133.6, 136.9, 141.2, 141.2, 143.2, 152.7, 159.6, 178.4 ; LC/ESI calculated for ($C_{23}H_{22}N_4O_2S$) m/z $[M + H]^+$418.15, found m/z 419.1155.

Synthesis of N-(4-(tosylamino) phenyl) indoline-1-carbothio amide (4a)

To a solution of compound 2 (0.4 g, 0.00150 mol) in 10 mL THF, pyridine (0.474 mL, 0.006 mol) was added at 0 ^{0}C followed by 4-toluene sulfonyl chloride (0.285 g, 0.00150 mol) in 2 mL 1,2-dichloroethane was added . The reaction mixture was allowed to RT and stirred for 3 h. The progress of the reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure and separated between ethyl acetate (50 mL) and water (50 mL). The combined ethyl acetate layer was washed with 2N HCl (1 x 30 mL), 10 % $NaHCO_3$ (2 x 25 mL), brine (2 x 50 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate layer was concentrated under reduced pressure to afford compound **4a**. White solid; Yield 48 % mp 220-222 ^{0}C; IR (KBr) ν_{max} in cm^{-1} : 3423 (NH stretch), 3282, 3059 (CH stretch, aromatic), 2921 (CH stretch, aliphatic), 1595 (NH bend), 1516 (CH_2 bend), 1371, 1154 (S=O), 749 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.34 (s, 3H), 3.08 (t, 2H, J = 8.4 Hz), 4.22 (t, 2H, J = 8.4 Hz), 6.95-7.09 (m, 3H), 7.11 (t, 1H, J = 7.8 Hz), 7.20-7.27 (m, 3H), 7.35 (d, 2H, J = 8.1 Hz), 7.64 (d, 2H, J = 8.1 Hz), 8.9 (d, 1H, J = 8.1 Hz), 9.63 (s, 1H), 10.18 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ ppm: 20.9, 26.6, 53.1, 116.0, 120.1, 123.0, 125.1, 125.66, 126.3, 126.6, 129.6, 133.6, 134.2, 136.2, 136.7, 142.8, 143.1, 178.1; LC/ESI calculated for ($C_{22}H_{21}N_3O_2S_2$) m/z $[M + H]^+$ 423.11, found m/z 424.0771.

Synthesis of N-(4-{[(2,6-dichlorophenyl)sulfonyl]amino}phenyl) indoline-1-carbothio amide (4b)

Prepared as reported above for 4a, starting from compound 2 and 2,6-dichlorobenzene-1-sulfonyl chloride. This reaction was carried out at room temperature for 3 h. Red solid; Yield 37 % ; mp 215-217 ^{0}C ; IR (KBr) ν_{max} in cm^{-1} : 3432 (NH stretch), 3360, 3099 (CH stretch, aromatic), 2922, 2850 (CH stretch, aliphatic), 1600 (NH bend), 1519 (CH_2 bend), 1387, 1165 (S=O), 747 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.09 (t, 2H, J = 8.1 Hz), 4.21 (t, 2H, J = 8.2 Hz), 7.04-7.07 (m, 2H), 7.14 (d, 1H, J = 8.5 Hz), 7.23-7.26 (m, 3H), 7.52-7.71 (m, 3H), 8.10 (d, 1H, J = 8.4 Hz), 9.63 (s,1H), 10.78 (s,1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 27.1, 53.7, 116.5, 119.6, 123.5, 125.6, 126.3, 126.8, 127.6, 130.7, 132.4, 133.7, 134.2, 134.92, 136.8, 143.4,178.7 ; LC/ESI calculated for ($C_{21}H_{17}Cl_2N_3O_2S_2$) m/z $[M + H]^+$ 477.01, found m/z 477.9745

Synthesis of 2-(1H-indol-3-yl)-N-(4-(indoline-1-carbothio amido) phenyl) acetamide (5a)

To a solution of compound 2 (0.4 g, 0.00150 mol) in 12 mL, 3-indole acetic acid (0.316 g, 0.00181 mol), EDC.HCl (0.370 g, 0.00195 mol) and HOBt (0.202 g, 0.00150 mol) was added. The mixture was cooled to 0 ^{0}C and triethylamine (0.818 mL, 0.006 mol) was added. The resulting solution was allowed to RT and stirred for 8 h. The reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure and resulting crude product was separated between ethyl acetate (50 mL) and water (50 mL). The combined ethyl acetate layer was washed with 2N HCl (1 x 30 mL), 10% $NaHCO_3$ (2 x 20 mL), brine (2 x 50 mL) and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure to afford compound **5a**. Pal yellow solid; Yield 40%; mp 243-245 ^{0}C; IR (KBr) ν_{max} in cm^{-1} : 3406 (NH stretch), 3310, 3184 (CH stretch, aromatic), 2904 (CH stretch, aliphatic), 1657 (C=O), 1527 (NH bend), 1482 (CH_2 bend), 1291 (C-N), 1254 (C=S), 788 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.09 (t, 2H, J = 8.1 Hz), 3.72 (s, 2H), 4.24 (t, 2H, J = 8.1 Hz), 6.97 (td, 2H, J = 1.2, 6.9 Hz), 7.0-7.15 (m, 2H), 7.28 (m, 4H), 7.35 (d, 1H, J = 8.1 Hz), 7.54 -7.62 (m,3H), 8.16 (d, 1H, J = 8.3 Hz), 9.67 (s, 1H), 10.12 (s, 1H), 10.92 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 33.7, 53.1, 108.6, 111.35, 116.0, 118.3, 118.6, 118.9, 120.9, 122.9, 123.8, 125.1, 126.3, 127.2, 133.6, 135.0, 136.1, 136.2, 143.1, 169.5, 178.4 ; LC/ESI calculated for ($C_{25}H_{22}N_4OS$) m/z $[M + H]^+$426.15, found m/z 427.1205.

***Synthesis of 3-fluoro-N-(4-(indoline-1-carbothio amido)phenyl)benzamide* (5b)**

Prepared as reported above for 5a, starting from compound 2 and 3-fluorobenzoic acid. This reaction was carried out at RT for 7 h. White solid; Yield 24 %; mp 220-222 ^{0}C; IR (KBr) ν_{max} in cm^{-1} : 3422 (NH), 3336, 3271 (CH stretch aromatic), 2930 (CH stretch aliphatic), 1657 (C=O), 1593 (NH bend), 1516 (CH_2 bend), 1388 (C-F), 1317 (C-N), 746 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.17 (t, 2H, J = 8.1 Hz), 4.27 (t, 2H, J = 8.4 Hz), 6.99 (t, 1H, J = 7.2 Hz), 7.15 (t, 1H, J = 8.1 Hz), 7.32 (d, 1H, J = 7.2 Hz), 7.36 (d, 2H, J = 8.7 Hz), 7.47 (t, 1H, J = 7.8 Hz), 7.56-7.63 (m, 1H), 7.71 (d, 2H, J = 8.5 Hz), 7.76-7.83 (m, 2H), 8.15 (d, 1H, J = 8.4 Hz), 9.75 (s, 1H), 10.33 (s,1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 53.2, 114.5, 116.0, 118.3, 120.3, 123.8, 125.1, 126.3, 130.5, 130.6, 133.7, 135.5, 135.8, 137.2, 143.0, 160.7, 163.1, 163.9, 178.4 ; LC/ESI calculated for ($C_{22}H_{18}FN_3OS$) m/z $[M + H]^+$ 391.12, found m/z 392.0887

***Synthesis of 2-(2-fluorophenyl)-N-(4-(indoline-1-carbothio amido) phenyl)acetamide* (5c)**

Prepared as reported above for 5a, starting from compound 2 and 2-fluoro phenylacetic acid. This reaction was carried out at RT for 7 h. Yellow solid; Yield 24 % ; mp 220-222 ^{0}C ; IR (KBr) ν_{max} in cm^{-1}: 3328 (NH stretch), 3289, 3066 (CH stretch, aromatic), 2632 (CH stretch, aliphatic), 1672 (C=O), 1600 (NH bend), 1510 (CH_2 bend), 1388 (C-F), 1317 (C-N), 744 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.10 (t, 2H, J = 8.4 Hz), 3.73 (s, 2H), 4.25 (t, 2H, J = 8.4 Hz), 6.97 (t, 1H, J = 6.6 Hz), 7.11-7.21 (m, 3H), 7.27 (d, 4H, J = 6.8 Hz), 7.31-7.42 (m, 1H), 7.54 (d, 2H, J = 8.6 Hz), 8.16 (d, 1H, J = 8.3 Hz), 9.69 (s, 1H), 10.23 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 36.2, 53.1, 115.1, 116.0, 118.9, 122.9, 123.0, 124.1, 125.1, 126.3, 128.7, 128.7, 131.9, 133.6, 135.2, 143.0, 159.4, 161.8, 167.8, 178.4 ; LC/ESI calculated for ($C_{23}H_{20}FN_3OS$) m/z $[M + H]^+$ 405.13, found m/z 406.0980.

***Synthesis of N-(4-(indoline-1-carbothio amido) phenyl)cyclopentanecarboxamide* (5d)**

Prepared as reported above for 5a, starting from compound 2 and cyclopentane carboxylic acid. This reaction was carried out at RT for 8 h. White solid; Yield 27 % ; mp 213-215 ^{0}C ; IR (KBr) ν_{max} in cm^{-1} : 3298 (NH stretch), 3040 (CH stretch, aromatic), 2928, 2860 (CH stretch, aliphatic), 1670 (C=O), 1602 (NH bend), 1518 (CH_2 bend), 1317 (C-N), 744 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.56-1.748 (m, 7H), 1.84 (d, 2H, J = 8.1 Hz), 3.10 (t, 2H, J = 8.1 Hz), 4.25 (t, 2H, J = 8.4 Hz), 6.98 (t, 1H, J = 7.5 Hz), 7.13 (t, 1H, J = 7.5 Hz), 7.27 (d, 3H, J = 8.4 Hz), 7.55 (d, 2H, J = 8.7 Hz), 8.16 (d, 1H, J = 8.1 Hz), 9.70 (s, 1H), 9.82 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.1, 27.2, 30.5, 45.7, 53.6, 116.5, 119.4, 123.43, 125.6, 125.8, 126.8, 134.1, 135.4, 136.8, 143.6, 174.6, 178.9 ; LC/ESI calculated for ($C_{21}H_{23}N_3OS$) m/z $[M + H]^+$ 365, found m/z 366.1384.

***Synthesis of N-(4-(indoline-1-carbothio amido) phenyl)acetamide* (5e)**

To a solution of compound 2 (0.4 g, 0.00150 mol) in 10 mL of THF, triethylamine (0.757 mL, 0.0075 mol) was added at 0 ^{0}C followed by acetyl chloride was added. The reaction mixture was allowed to RT and stirred for 1 h. The reaction was monitored by TLC. The reaction mass was concentrated under reduced pressure and separated between ethyl acetate (50 mL) and water (50 mL). The combined ethyl acetate layer was washed with 2N HCl (1 x 25 mL), 10 % $NaHCO_3$ (2 x 20 mL), brine (2 x 50 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate layer was concentrated under reduced pressure to afford compound 5e, white solid; Yield 42 % mp 200-202 ^{0}C; IR (KBr) ν_{max} in cm^{-1} : 3428 (NH stretch), 3303, 3044 (CH stretch, aromatic), 2928 (CH stretch, aliphatic), 1661 (C=O), 1600 (NH bend), 1517 (CH_2 bend), 1315 (C-N), 743 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.21 (s, 3H), 3.10 (t, 2H, J = 8.4 Hz), 4.25 (t, 2H, J = 8.4 Hz), 6.9 (t, 1H, J = 7.5 Hz), 7.14 (t, 1H, J = 7.5 Hz), 7.27 (d, 3H, J = 8.7 Hz), 7.52 (d, 2H, J = 8.7 Hz), 8.16 (d, 1H, J = 8.5 Hz), 9.67 (s, 1H), 9.94 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 23.9, 26.5, 116.0, 118.8, 122.9, 125.4, 126.3, 133.6, 134.9, 136.1, 143.0, 168.0, 178.4 ; LC/ESI calculated for ($C_{17}H_{17}N_3OS$) m/z $[M + H]^+$ 311.11, found m/z 312.0977.

PHARMACOLOGY

In vitro antibacterial study

Anti-bacterial activity of all the synthesized compounds was carried by using agar well diffusion method[18] at a concentration of 100µg/mL and compare with standard chloramphenicol. The test compounds were evaluated anti-bacterial activities against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia.* The Standard antibiotic chloramphenicol (30 µg) was used as positive control for each bacterial species tested. Then the inoculated plates were incubated at 37 ^{0}C for 24 h. At the end of incubation period the zone of inhibition was measured diameter in millimetre as its anti bacterial activity.

In vitro antifungal study

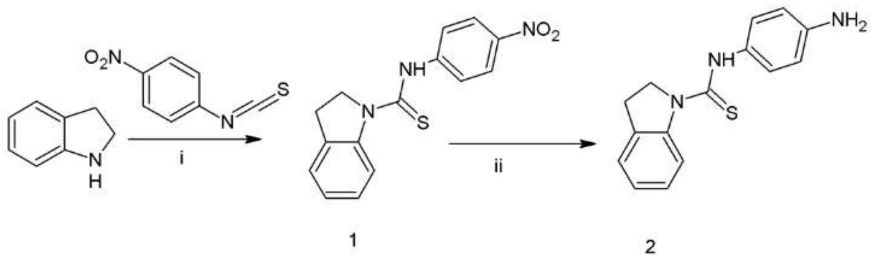
Potato dextrose agar (PDA) was used for fungal cultures. The cultures medium was inoculated with the fungal strains separately suspended in potato dextrose broth. The synthesized compounds were injected to sterile disc. The standard drug fluconazole (30 µg) was used as positive control and fungal plates were incubated in 37 ^{0}C for 72 h. At the end of incubation period the zone of inhibition was measured diameter in millimetre as its anti fungal activity.

RESULTS AND DISCUSSION

Chemistry

The synthetic scheme for the preparation of intermediates and final compounds are shown in scheme **(1-3).** The intermediate-1 was prepared[19] by using the reagent 4-nitrophenyl isothiocyanate. The FT-IR spectrum of intermediate-1 had characteristic NH absorption peaks at 3434 cm^{-1} and 3300 cm^{-1}. The absorption peaks for C=S seen at 1270 cm^{-1}. The NO_2 stretching frequency observed at 1319 cm^{-1} and 1532 cm^{-1}. In the ^{1}H-NMR spectra of compound-I, the proton signal of thiourea NH was observed at δ 10.39 (1H, singlet). Characteristic peaks of thiocarbamide compound were observed at δ 10.39 ppm. In ^{13}C-NMR of intermediate-2, the characteristic peaks at thiocarbamide C=S resonate at 177.5 ppm. The intermediate-2 was prepared[20] by reduction of intermediate-1 by $SnCl_2$. The FT-IR spectra of intermediate-2 showed absorption band at 3398 cm^{-1} due to NH stretching vibration from resulting amine group formed. In the FT-IR spectrum of intermediate-2, the reduction of NO_2 group was confirmed by disappearance of NO_2 stretching vibration at 1319 cm^{-1} and 1532 cm^{-1}. In the ^{1}H-NMR spectra of intermediate-2, the characteristic amine proton peak seen as broad singlet at δ 5.05 ppm. The resulting compound was also confirmed by TLC, which was exhibited ninhydrin activity. The intermediate-2 was taken a common scaffold for synthesis of proposed indoline derivatives. The synthetic path way for intermediate-2 is outlined below.

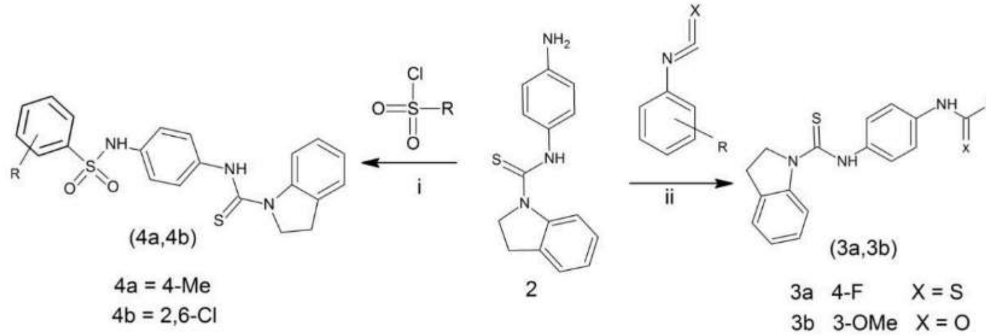
Scheme 1



Scheme 1: Reagents and conditions: (i). 4-nitrophenyl isothiocyanate, THF, 0 ^{0}C; (ii) $SnCl_2$, 12N HCl, RT , 0 ^{0}C.

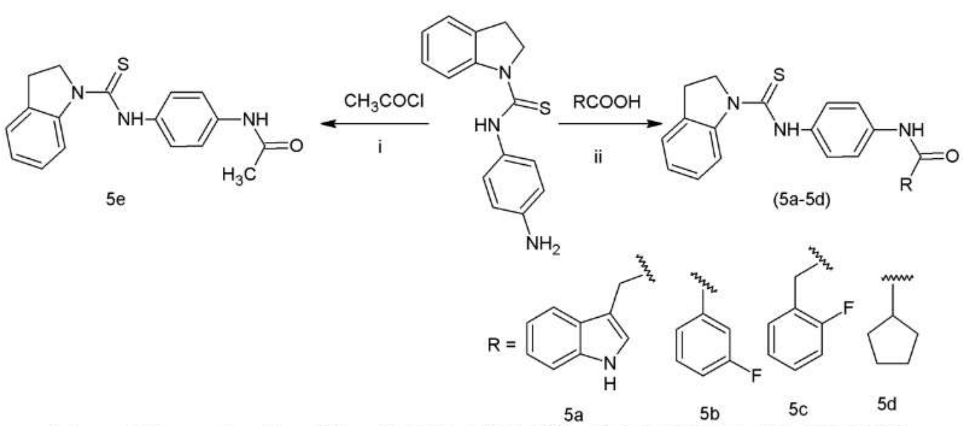
The sulfonamide derivates 4a and 4b were prepared[21] by reaction between corresponding sulfonyl chloride and key intermediate-2. The reaction was carried out in dichloroethane with pyridine base at 90 ^{0}C. The FT-IR spectrum resulted in sulfonamide derivatives exhibited absorption wavelength at 1154 cm^{-1} and 1387 cm^{-1}. Which is the characteristic peak of (S=O) group. The ^{1}H-NMR spectra of synthesized sulfonamide derivatives showed new singlet peak at δ 9.63 ppm.

The desired title compounds **3a** and **3b** were obtained in resonable yield[22] by treating key intermediate-2 with corresponding isocyanate and isothiocyanate respectively. In the FT-IR spectrum of **3a** characteristic C=S seen at 1211 cm^{-1} while in **3b** characteristic C=O 1649 cm^{-1}. In the ^{1}H-NMR of 3a, NH proton of thiourea group resonated at δ 9.78 ppm. In the case of 3b, NH proton of new urea group resonated at δ 9.67 ppm. According to ^{13}C-NMR of 3a and 3b, the compounds has displayed characteristic thiourea carbonyl carbon C=S at δ 179.9 ppm and urea carbonyl carbon C=O at δ 159.6 ppm. The synthetic scheme for preparation of sulfonaminde, urea and thiourea derivatives are shown in **Scheme 2.**



Scheme 2: Reagents and conditions (i). Pyridine, Dichloroethane, 90 ^{0}C. (ii). THF, 0 ^{0}C.

From the above key intermediate-2, the carboxamide derivatives (**5a-d**) were prepared[23] by using reagent 1-Ethyl -3-(3-dimethylaminopropyl) carbodiimide (EDCI). The corresponding acid chloride was used for preparing[24] the amide derivatives 5e. The FT-IR spectrum of all the carboxamide derivatives has strong C=O absorption, which showed wave length between 1657-1672 cm^{-1}. The ^{1}H-NMR spectra of all carboxamide derivatives exhibited broad signals at δ 9.7-10.1ppm is assigned to NH. In addition, ^{13}C-NMR spectra of all carboxamide derivatives displayed signals at δ 163.9-174.6 ppm. All the FT-IR, ^{1}H-NMR and mass spectral data of compounds (**5a-e**) were in accordance with the proposed molecular structures. The purity of synthesized compounds was monitored by TLC. The synthetic scheme for the preparation of all carboxamide derivatives is depicted in **Scheme-3.**



Scheme 3: Reagents and conditions (i). TEA, MDC, 0 ^{0}C., (ii). EDCI, HOBt, TEA, THF, 0 ^{0}C;

In vitro Antibacterial Evaluation

The antibacterial activity of all the synthesized compounds was carried out by using disc well diffusion method at a concentration of (100 µg/ml) and compared with known standard drug chloramphenicol. From the zone inhibition result, the indoline derivatives displayed moderate to high activity against all the tested strains. Like compounds 5a, 5b, 5c, and 5e exhibited significant activity against *Escherichia coli* and *Klebsiella pneumonia*, the most of the compounds like 4a, 4b and 5d showed moderate activity. Remaining compounds did not show any noticeable activity. The zone of inhibition of all compounds is listed in table 1.

In vitro Antifungal Evaluation

The antibacterial activity of all the synthesized compounds was carried out by using disc well diffusion method at a concentration of (100µg/ml) and compared with known standard drug fluconazole. From the result data, the compounds 5b and 5e showed good activity against two fungal strains *Candida albican* and *Candida glabrata* remaining the compounds did not show any markable activity. The zone of inhibition of all compounds is summarized in table 1.

Table 1: Anti-microbial activity of indoline derivatives

Compound code	Zone of inhibition (diameter in mm)					
	Anti-bacterial				Anti-fungal	
	SA	BS	EC	KP	CA	CG
3a	-	-	-	-	-	-
3b	-	-	-	-	-	-
4a	8	9	7	9	-	-
4b	9	6	8	7	9	7
5a	9	8	19	20	8	-
5b	7	9	**23**	**22**	**13**	8
5c	9	7	21	20	9	7
5d	10	12	14	13	8	6
5e	9	7	20	19	11	-
chloramphenicol	26	27	26	28	-	-
fluconazole	-	-	-	-	27	26

SA=*Staphylococcus aureus* (MTCC-2940), BS= *Bacillus Subtilis* (MTCC-441), EC=*Escherichia coli* (MTCC-739), *Klebsiella pneumonia* (ATCC-10031), CA= *Candida albican* (ATCC-2091). CG= *Candida glabrata*(ATCC-36583).

CONCLUSION

It could be concluded that the synthesized indoline compounds were evaluated for their in *vitro* anti-microbial activity. The target compounds **5a, 5b, 5c** and **5e** showed good activity. Based on the above findings the novel indoline derivatives **5a, 5b, 5c** and **5e** selected for future optimization and development in animal models for detailed efficacy studies.

ACKNOWLEDGEMENT

The authors are very much thankful to Dr.R.S.D. Wahida Banu, Principal, Government College of Engineering Salem-11 and gratefully acknowledged.

REFERENCES

1. Virtonean A I and Hietala P K. Acta Chemica Scandinavica 1960; 14: 499-502.
2. Milind A, Rode, Sahebrao s, Rindhe and Bhausaheb K, Karale. Synthesis and biological activites of some indoline derivatives. Journal of the Serbian chemical Society 2009; 74: 1377-1387.
3. Mondal P, Jana S, Balaji A, Ramakrishna R, Kanthal LK. Synthesis of some new isoxazoline derivatives of chalconised indoline 2-one as a potential analgesic, antimicrobial and anthelmimtic agents journal of Young pharmacists 2012; 4:38-41.
4. Nandakumar A, Prakasam Thirumurugan, Paramasivan T, Perumal, P. Vembu M.N. Ponnuswamy. One-pot multicomponets synthesis and anti-microbial evaluation og 2-(indol-3-yl)-2-oxospiro (indoline-3,4-pyran) derivatives. Bioorganic & Medicinal Chemistry Letters 2010; 20: 4252-4258.
5. Abdel Rahman A H, Keshk E M, Hanna M A and Sh, El Bady M. Synthesis and evaluation of some new spiro indoline-based hetrocycles as potentially active antimicrobial agents. Bioorganic&Medicinal Chemistry 2004; 12**:** 2483- 2488.
6. Girija S, Singh, Patrick Luntha. Synthesis and antimicrobial activity of new 1-alkyl/cyclohexyl-3,3-diaryl-1-methyl spiro[azetidine -2,3-indoline]-2,4-diones.2009 European Journal of Medicinal Chemistry 2009; 44**:** 2265- 2269.
7. Irena Sovic Sandra Kraljevic PavelicElitza Markova Carb, Natasa Ilic Raja Nhilic, Sabine Depauw, Marie Helene David Cordonnier and Grace Karminski Zamola. Novel phenyl and pyridyl substituted serivatives of isonidolines: synthesis, antitumor activity and DNA binding features. European Journal of Medicinal Chemistry 2014; 87: 372-385.
8. Pengzhan Li, Yanmei Tan, Guyue Liu, Yang Liu, Jianzhen Liu, Yanzhen Yin, Guisen Zhao, Synthesis and biological evaluation of novel indoline-2,3-derivatives as antitumor agents, Drug Discoveries & Therapeutics 2014; 8: 110- 116.
9. Xiao Liang Xu, Chun Lei Yu, Wen Chen, Ying Chao Li, Juan Yang Li, Yan Li, Hong BinZhang, Xiao Dong Yang, Synthesis and Antitumor Activity of novel 2- substituted indoline imidazolium salt derivatives, Organic Biomolecular Chemistry2005; 13: 1550 -1557.
10. Kang Jin, Xiaopan Zhang, Chunhun Ma, Yingying Xu, Yumei Yuan, Wenfang Xu. Novel indoline-2,3-dione derivatives as inhibitors of aminopeptidase N (APN).Bioorganic & Medicinal Chemistry 2013; 21: 2663-2670.
11. Kenjiro Sato, Hiromichi Sugimoto, Kentaro Rikimaru, Hiroshi Imoto, Masahiro Kamaura, Nobuyuki Negoro, Yoshiyuki Tsujihata, Hirohisa Miyashita, Tomoyuki Odani, Toshiki Murata. Discovery of a novel series of indoline carbamate and indolinylpyrimididine derivatives as potent GPR119 agonists. Bioorganic & Medicinal Chemistry 2014; 22: 1649-1666.

12. Svetiana Furman, Elinor Nissim Bardiugo, Shani Zeeli, Michal Weitman, Abraham Nudelman, Efrat Finkin Gorner, Dorit Moradov, Helena Shifrin, Donna Schorer Apelbaum, Marta Weinstock, Synthesis and in vitro evaluation of anti-inflammatory activity of ester and amine derivatives of indoline in RAW 264.7 and peritoneal macrophages. Bioorganic & Medicinal Chemistry Letters 2014; 24: 2283-2287.
13. Dhineshkumar Manoharan, Kannan kulanthi and Gnanavel Sadhasivam, Synthesis, Characterization and Evaluation of Anti-inflammatory Activity of Novel Indoline Derivatives. Journal of Advances in chemistry 2016; 12: 4557-4563.
14. Jang Yang Chang, Hsing Pang Hsieh, Chi Yen Chang, Kuo Shun Hsu, Yi Fang Chiang, Chi Ming Chen, Ching Chuan Kuo and Jing Ping Liou. 7-Aryl-aminoindoline-1-sulfonamides as a Novel Class of Potent Antitubulin Agents. Journal of Medicinal Chemistry 2006; 49: 6656-6659.
15. Shoji Kamiya, Hiroaki Shirahase, Akihisa Yoshimi, Shohei Nakamura, Mamoru Kanda, Hiroshi Matsui, Masayasu Kasai, Kenji Takashi and Kauyoshi Kurahashi. Bioavailable acyl-CoA : Cholesterol Acyltransferase Inhibitor with Anti-peroxidative Activity: Synthesis and Biological activity of Novel Indoline Amide and Urea Drivatives. Chem.Pharm.Bull .2000; 48: 817- 827.
16. Tetsuji Noguchi, Naoki Tanaka, Toyaki Nishimata, Riki Goto, Miho Hayakawa, Atsuhiro Sugidachi, Taketoshi Ogawa, Fumitoshi Asai, Tomoko Ozeki and Kochi Fujimoto. Indoline derivatives II: Synthesis and factor Xa (FXa) inhibitory activities. Chemical Pharmaceutical Bullatin 2007; 55: 393-402.
17. Dan Zhang, Hao Song and Young Qin. Total Synthesis of Indoine Alkaloids: A Cyclopropanation Strategy. Accounts of chemical research 2011; 44**:** 447-457.
18. Bauer A W, Kirby W M, Sherris J C, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 1966; 45:493-496.
19. Sidoova, E Kralova, K & Loss, D. Synthesis of 2-(6-acetamido benzo thiozolethio) acetic acid ester as photosynthesis inhibitor. Moleules 1998; 3: 135-140.
20. Katritzky, AR & Rachwal, S. New routes to selectively methylated benzimidazoles. Journal of Heterocyclic Chemistry 1994; 31:775-779.
21. Benigno, M, Isabel, G, Maria, VV, Joaquın, B & Francisca, S. Synthesis and characterization of sulfonamides containing 8-aminoquinoline and their Ni(II) complexes Crystalline structure of the Ni complexes. Crystalline structures of the Ni complexes. Polyhedron 2002; 21: 1229-1234.
22. Zhili, X, Hongyu, Z, Michael, S, Bruce, GS, Harriet, TS, Tom, SS & Rich, SJ. Synthesis and biological evaluation of N-Formyl hydroxylamine derivatives as potent peptides deformylase inhibitors. Bioorganic Medicinal Chemistry Letters 2008; 18:4298-302.
23. Jing, X, Hai-Feng, Z, Ya-Juan, Z, Yun-Jun, L, Ji-Wang, J, Jing-Jing, Y & Shu-Feng, Z. Synthesis and antitumor activity of amino acid ester derivatives containing 5-Fluorouracil. Molecule 2009; 14: 3142-52.
24. Seung-Woo, Y, Hee-Yeol, L, Bong-Hwan, C, Kyung-Mi, A, Jung-Su, R, Young-Ho, L & Jae-Hoon, K. Synthesis and biological evaluation of N-formyl hydroxylamine derivatives as potent peptide deformylase inhibitors. Bullatin Korean Chemical Society 2006; 27:1075-79.

Cite this article as:

Dhineshkumar Manoharan *et al*. Synthesis, characterization and *In vitro* antimicrobial evaluation of Indoline based derivatives. Int. Res. J. Pharm. 2017;8(4):132-137 http://dx.doi.org/10.7897/2230-8407.080463

Source of support: Nil, Conflict of interest: None Declared